SUBGROUP: Molecular Biophysics

1-Subg
Precise Mapping of RNA Tertiary Structure via Nanometer Distance Measurements with Double Electron-Electron Resonance (DEER) Spectroscopy
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Dynamic RNA structural changes are critical in biology, and a continuing challenge is to develop methods for precision mapping of these structures. Inter-label distance measurements by DEER (Double-Electron Electron Resonance) spectroscopy in conjunction with side-directed RNA spin labeling provide precise nanoscale structural constraints for biomolecules. This technique has been demonstrated in helical and hairpin RNAs, but has rarely been applied to complex RNA structures. We have used DEER to monitor a large-scale Mg^{2+}-triggered RNA folding transition in the Hammerhead ribozyme, a three-helix junction motif that undergoes an inactive-to-active structural change upon addition of Mg^{2+}. A distinct increase in the population of ribozymes is observed with a short inter-label distance with increasing [Mg^{2+}]. The measured inter-label distance is remarkably consistent with models generated from static crystal structures when it is assumed that the spin labels preferentially localize near to the RNA minor grooves. Data from labels located close to the catalytic core of the ribozyme will probe a putative local structural change in this RNA that may be linked to its catalytic activity. The DEER technique described here can be applied to predict folding of other functional RNA molecules, including those found in complex RNA-protein complexes.
1. Kim, N.-K.; Bowman, M.K.; DeRose, V.J. ''Precise Mapping of RNA Tertiary Structure via Nanometer Distance Measurements with Double Electron-Electron Resonance Spectroscopy (DEER) Spectroscopy'' *J. Amer. Chem. Soc.* **2010**, *132*, 8882-8884.

2-Subg
Molecular Insights Into the Organization and Folding Dynamics of Metabolite-Sensing Riboswitches
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Riboswitches are naturally-occurring genetic elements that regulate gene expression usually by binding to specific cellular metabolites. They are composed of two modular domains that correspond to the aptamer and the expression platform. The aptamer domain is the most conserved region of the riboswitch as it involved in the specific recognition of the ligand. The expression platform is less conserved and can vary to a high degree in its structure given that it regulates at various biological levels, such as transcription, translation and splicing. Using single-molecule FRET together with a combination of biochemical and biophysical assays, we have studied the relationship between aptamer structure, folding and activity in two members of the riboswitch family: the S-adenosylmethionine (SAM) and the adenine riboswitch (A-box).We will propose a folding pathway for each riboswitch aptamer in the presence and absence of their cognate metabolite and we will demonstrate that the role played by the ligand is fundamentally different in both riboswitch classes. For the adenine aptamer, ligand recognition induces only very subtle changes in the folding dynamics and the local architecture of the binding site. On the contrary, binding of the SAM ligand controls the final organization of the SAM aptamer promoting major conformational changes in the aptamer architecture. Our findings provide experimental evidences for a classification of riboswitches according to the degree in which ligand-recognition events control the aptamer folding pathway.

3-Subg
Insights Into the Dynamic Personalities of Synthetic Riboswitches by NMR-Spectroscopy
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Riboswitches are novel gene regulatory elements found in the 5'- and 3'-untranslated regions of mRNAs. They regulate gene expression upon direct and specific interaction with small molecule ligands. In most riboswitches ligand binding triggers conformational changes on the secondary and/or tertiary structure level which result in changes in gene expression levels. While a lot is known about the structural basis for ligand recognition by riboswitch domains the conformational dynamics of riboswitches which are central to their function is less explored. NMR-spectroscopy in solution is well suited for investigating dynamical systems and for characterising conformational changes. We applied NMR-spectroscopy to two smaller synthetic riboswitches to characterize in detail their conformational dynamics in relation to their regulatory functions.

4-Subg
Accurate Single-Molecule FRET Studies of Nucleic Acids Using Multi-Parameter Fluorescence Detection
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So far our view of protein function is formed, to a significant extent, by traditional structure determination showing many beautiful static protein structures. Recent experiments by single-molecule and other techniques have questioned the idea that proteins and other biomolecules are static structures. We used multi-parameter fluorescence detection (MFD) to perform smFRET studies of free diffusing biomolecules. We demonstrate that the simultaneous acquisition of most fluorescent parameters by MFD allows for a robust assessment of all possible artefacts involved in single-molecules FRET and offers unsurpassed capabilities regarding the identification and analysis of individual species present in population of molecules [1]. A toolbox is introduced in order to demonstrate how complications originating from orientation, mobility and position of fluorophores and conformational dynamics [2] have to be taken into account when determining FRET related distances with high accuracy. Although static structures are known for many biomolecules, the functions of biomolecules are governed ultimately by their dynamic character. In this view we give various examples of smFRET experiments of DNA- and RNA-junctions. Their Mg-dependent structural dynamics is studied in detail. These studies show that smFRET studies are valuable tool to complement the structural and dynamic information obtained by X-ray crystallography or NMR spectroscopy.
[1] Sisamakis, E., Valeri, A., Kalinin, S., Rothwell, P. J., **Seidel, C. A. M.**; *Accurate single-molecule FRET studies using multiparameter fluorescence detection*. Methods in Enzymology **475** (Single Molecule Methods, Part B: Multiparameter, super-resolution, tethering, and force based methods, Ed. Nils Walter) Chapter 18, 455-514 (2010).
[2] Kalinin, S., Valeri A., Antonik M., Felekyan, S., **Seidel, C. A. M.**; *Detection of structural dynamics by FRET: A photon distribution and fluorescence lifetime analysis of systems with multiple states.* J. Phys. Chem. B. **114**, 7983-7995 (2010).

5-Subg
The Paths to Specific vs Nonspecific Sequence Recognition
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Our understanding of both nonspecific and specific protein-DNA interaction mechanisms raise questions related to the nature of the transient encounter states, and the structural and energetic features of the protein-DNA binding process. Here we have used the nuclease domain of colicin E7 (N-ColE7) from E. coli to complex with a 12-bp DNA as the model system to draw a picture of how a protein approaches, encounters and associates with DNA. Multiscale studies using Molecular Dynamics (MD) and Brownian Dynamics (BD) simulations were performed to provide the binding process on multiple length and timescales. We characterize the encounter states and identify the spatial and orientational aspects required for the association by BD simulations. At the atomic length-scales, we investigated the binding process by MD simulations. Several intermediate binding states, which have different positions and orientations of protein around DNA in the initial structures are postulated from the MD trajectories. The results facilitate better understanding of sequence-independent protein-DNA binding landscapes and suggest pathways with favorable intermediate binding states common to both specific and nonspecific complexes.

6-Subg
RNA Structure, Function, and (Thermo-) Dynamics: A SAXS and Single-Molecule Perspective
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Nucleic acids are central to the storage, transmission, and control of genetic information. Their cellular function is dependent not only on sequence, but also on their three-dimensional shape, mechanical properties, and conformational dynamics. Here, we discuss three recent developments that have provided unique insights into these important aspects of DNA and RNA functions.
Firstly, small-angle X-ray scattering (SAXS) is emerging as an important tool to study RNA structure. We have demonstrated that SAXS can provide ab initio low-resolution 3D models of RNAs [1]. By taking into account prior